

The present invention describes a method to scan gene databases for the simple active site motif characteristic of aspartyl proteases. Eukaryotic aspartyl proteases such as pepsin and renin possess a two-domain structure which folds to bring two aspartyl residues into proximity within the active site. These are embedded in the short tripeptide motif DTG, or more rarely, DSG. Most aspartyl proteases occur as proenzyme whose N-terminus must be cleaved for activation. The DTG or DSG active site motif appears at about residue 65-70 in the proenzyme (prorenin, pepsinogen), but at about residue 25-30 in the active enzyme after cleavage of the N-terminal prodomain. The limited length of the active site motif makes it difficult to search collections of short, expressed sequence tags (EST) for novel aspartyl proteases. EST sequences typically average 250 nucleotides or less, and so would encode 80-90 amino acid residues or less. That would be too short a sequence to span the two active site motifs. The preferred method is to scan databases of hypothetical or assembled protein coding sequences. The present invention describes a computer method to identify candidate aspartyl proteases in protein sequence databases. The method was used to identify seven candidate aspartyl protease sequences in the *Caenorhabditis elegans* genome. These sequences were then used to identify by homology search Hu-Asp1 and two alternative splice variants of Hu-Asp2, designated herein as Hu-Asp2(a) and Hu-Asp2(b).

In a major aspect of the invention disclosed here we provide new information about APP processing. Pathogenic processing of the amyloid precursor protein (APP) via the A β pathway requires the sequential action of two proteases referred to as β -secretase and γ -secretase. Cleavage of APP by the β -secretase and γ -secretase generates the N-terminus and C-terminus of the A β peptide, respectively. Because over production of the A β peptide, particularly the A β ₁₋₄₂, has been implicated in the initiation of Alzheimer's disease, inhibitors of either the β -secretase and/or the γ -secretase have potential in the treatment of Alzheimer's disease. Despite the importance of the β -secretase and γ -secretase in the pathogenic processing of APP, molecular definition of these enzymes has not been accomplished to date. That is, it was not known what enzymes were required for cleavage at either the β -secretase or the γ -secretase cleavage site. The sites themselves were known because APP was known and the A β ₁₋₄₂ peptide was known, see US 5,766,846 and US 5,837,672, (incorporated by reference, with the exception to reference to "soluble" peptides). But what enzyme was involved in producing the A β ₁₋₄₂ peptide was unknown.

5 The present invention involves the molecular definition of several novel human
aspartyl proteases and one of these, referred to as Hu-Asp-2(a) and Hu-Asp2(b), has been
characterized in detail. Previous forms of asp1 and asp 2 have been disclosed. *see* EP
0848062 A2 and EP 0855444A2, inventors David Powel et. al., assigned to Smith Kline
10 Beecham Corp. (incorporated by reference) Herein are disclosed old and new forms of
Hu-Asp 2. For the first time they are expressed in active form, their substrates are
disclosed, and their specificity is disclosed. Prior to this disclosure cell or cell extracts were
required to cleave the β -secretase site, now purified protein can be used in assays, also
15 described here. Based on the results of (1) antisense knock out experiments, (2) transient
transfection knock in experiments, and (3) biochemical experiments using purified
20 recombinant Hu-Asp-2, we demonstrate that Hu-Asp-2 is the β -secretase involved in the
processing of APP. Although the nucleotide and predicted amino acid sequence of Hu-
Asp-2(a) has been reported, *see* above, *see* EP 0848062 A2 and EP 0855444A2, no
functional characterization of the enzyme was disclosed. Here the authors characterize the
25 Hu-Asp-2 enzyme and are able to explain why it is a critical and essential enzyme required
in the formation of $A\beta_{1-42}$, peptide and possible a critical step in the development of AD.

 In another embodiment the present invention also describes a novel splice variant of
Hu-Asp2, referred to as Hu-Asp-2(b), that has never before been disclosed

30 In another embodiment, the invention provides isolated nucleic acid molecules
comprising a polynucleotide encoding a polypeptide selected from the group consisting of
human aspartyl protease 1 (Hu-Asp1) and two alternative splice variants of human aspartyl
35 protease 2 (Hu-Asp2), designated herein as Hu-Asp2(a) and Hu-Asp2(b). As used herein, all
references to "Hu-Asp2" should be understood to refer to both Hu-Asp2(a) and Hu-Asp2(b).
Hu-Asp1 is expressed most abundantly in pancreas and prostate tissues, while Hu-Asp2(a)
25 and Hu-Asp2(b) are expressed most abundantly in pancreas and brain tissues. The invention
also provides isolated Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) polypeptides, as well as
40 fragments thereof which exhibit aspartyl protease activity.

 The predicted amino acid sequences of Hu-Asp1, Hu-Asp2(a) and Hu-Asp2(b) share
45 significant homology with previously identified mammalian aspartyl proteases such as
pepsinogen A, pepsinogen B, cathepsin D, cathepsin E, and renin. P.B.Szecs, *Scand. J. Clin.*
30 *Lab. Invest.* 52:(Suppl. 210 5-22 (1992)). These enzymes are characterized by the presence of
a duplicated DTG/DSG sequence motif. The Hu-Asp1 and HuAsp2 polypeptides disclosed

herein also exhibit extremely high homology with the ProSite consensus motif for aspartyl proteases extracted from the SwissProt database.

The nucleotide sequence given as residues 1-1554 of SEQ ID NO:1 corresponds to the nucleotide sequence encoding Hu-Asp1, the nucleotide sequence given as residues 1-1503 of SEQ ID NO:3 corresponds to the nucleotide sequence encoding Hu-Asp2(a), and the nucleotide sequence given as residues 1-1428 of SEQ ID NO:5 corresponds to the nucleotide sequence encoding Hu-Asp2(b). The isolation and sequencing of DNA encoding Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) is described below in Examples 1 and 2.

As is described in Examples 1 and 2, automated sequencing methods were used to obtain the nucleotide sequence of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b). The Hu-Asp nucleotide sequences of the present invention were obtained for both DNA strands, and are believed to be 100% accurate. However, as is known in the art, nucleotide sequence obtained by such automated methods may contain some errors. Nucleotide sequences determined by automation are typically at least about 90%, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of a given nucleic acid molecule. The actual sequence may be more precisely determined using manual sequencing methods, which are well known in the art. An error in sequence which results in an insertion or deletion of one or more nucleotides may result in a frame shift in translation such that the predicted amino acid sequence will differ from that which would be predicted from the actual nucleotide sequence of the nucleic acid molecule, starting at the point of the mutation. The Hu-Asp DNA of the present invention includes cDNA, chemically synthesized DNA, DNA isolated by PCR, genomic DNA, and combinations thereof. Genomic Hu-Asp DNA may be obtained by screening a genomic library with the Hu-Asp2 cDNA described herein, using methods that are well known in the art, or with oligonucleotides chosen from the Hu-Asp2 sequence that will prime the polymerase chain reaction (PCR). RNA transcribed from Hu-Asp DNA is also encompassed by the present invention.

Due to the degeneracy of the genetic code, two DNA sequences may differ and yet encode identical amino acid sequences. The present invention thus provides isolated nucleic acid molecules having a polynucleotide sequence encoding any of the Hu-Asp polypeptides of the invention, wherein said polynucleotide sequence encodes a Hu-Asp polypeptide having the complete amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or fragments thereof.